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Clinical Application of the Molecular Diagnosis of Spinal Muscular Atrophy: Deletions of Neuronal Apoptosis Inhibitor Protein and Survival Motor Neuron Genes

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The molecular genetic diagnosis of spinal muscular atrophy (SMA) has recently been complicated by the identification of two candidate genes, which are often deleted in affected individuals but are also occasionally deleted in apparently unaffected carriers. We present a compilation of genotypes, from our laboratory and recent reports, for the survival motor neuron (SMN) and neuronal apoptosis inhibitor protein (NAIP) genes. Bayesian analyses were used to generate probabilities for SMA when deletions are present or absent in SMN. We found that when the SMN^T exon 7 is deleted, the probability of SMA can reach greater than 98% in some populations, and when SMN^T is present, the probability of SMA is approximately 17 times less than the prior population risk. Deletion of NAIP exon 5, as well as SMN^T exon 7, is associated with a 5-fold increased risk of type I SMA. Case studies are used to illustrate differing disease risks for pre- and postnatal testing, depending on the presence of information about clinical status or molecular results. These analyses demonstrate that deletion screening of candidate genes can be a powerful tool in the diagnosis of SMA. Am. J. Med. Genet. 69: 159–165, 1997. © 1997 Wiley-Liss, Inc.

KEY WORDS: SMA; SMN; NAIP; disease risk calculations

INTRODUCTION

The direct application of molecular genetic findings to a clinical situation is not always possible. One condition that presents particular problems in the interpretation of molecular results is spinal muscular atrophy (SMA), one of the most common inherited causes of pediatric mortality. There are three types of this autosomal recessive disorder which are distinguished on the basis of age of onset and severity [Zerres and Schöneborn, 1995]. Type I SMA (Werdnig-Hoffmann) is acute and fatal, with an onset in the first 6 months and death before 2 years of age. Type II SMA is less severe with clinical signs appearing before 18 months of age: the infant is able to sit unaided, but never walks, and death usually occurs beyond age 2 years. Type III SMA (Kugelberg-Welander) is the mildest form of this disease with variable age of onset (after 18 months); the patients learn to walk unaided, and death occurs in adulthood.

Two candidate genes that may have direct involvement in the pathogenesis of SMA [Lefebvre et al, 1995; Roy et al., 1995] have been identified. One candidate, the gene for neuronal apoptosis inhibitory protein (NAIP), shows homology with a baculoviral inhibitor of apoptosis [Roy et al., 1995] and inhibits apoptosis in mammalian cells [Liston et al., 1996]. Absence of NAIP exon 5, or exons 5 and 6 (ΔNAIP) was found in approximately 45% of individuals with type I SMA and 18% of those with type II and III SMA. The other candidate gene, survival motor neuron (SMN), shows no strong homology with previously identified sequences, but has a greater association with all 3 types of SMA. Absence of the telomeric copy of SMN exon 7, or 7 and 8 (ΔSMN^T) was found in 98.7% of all SMA patients tested [Lefebvre et al., 1995].

Following the identification of NAIP and SMN, additional prevalence data for the deletions in these genes in SMA patients [Rodrigues et al., 1995; Cobben et al., 1995; Hahnen et al., 1995; Burlet et al., 1996] have

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largely confirmed the original findings. However, in addition to the deletions in affected individuals, some carrier parents and unaffected sibs of patients were also found to be homozygous for deletions of NAIP or SMN^T [Roy et al., 1995; Cobben et al., 1995; Hahnen et al., 1995; Burlet et al., 1996; Wang et al., 1996]. Despite these apparent contradictions, deletions of NAIP and particularly of SMN^T are so common in SMA patients and so rare in controls, that a utility of deletion screening of these genes as diagnostic markers for the presence of SMA has been suggested [Schöneborn et al., 1996]. In an effort to assess the diagnostic potential of deletion screening, we calculate here the risk of SMA in homozygous ΔSMN^T hypotonic infants, offspring of one or two SMA carrier parents, and offspring of parents from the general population. The presence of ΔNAIP in addition to ΔSMN^T is found to increase the relative risk of type I SMA. These findings are applied to several clinical cases that presented for molecular analysis.

METHODS

Mutation Testing

A population of 50 type I and 94 type II/III families with SMA-affected individuals from across Canada, but primarily Eastern Ontario and Western Quebec was screened for the presence of NAIP and SMN^T deletions. Individuals were tested for NAIP exon 5 deletions (ΔNAIP) by a modification of a previously reported technique [Roy et al., 1995]. Direct polymerase chain reaction (PCR) amplification of a 76 base pair sequence within exon 5 was multiplexed with amplification of amelogenin sequences (107 and 113 base pairs) as positive controls [Nakahori et al., 1991]. Primers homologous to NAIP exon 5 (CCGCTGGGTTTTACTTCACTG/CCGGCACCAAAGAGGATTAG) were designed to amplify a small fragment of DNA, thereby reducing the risk of spurious non-amplification with degraded samples.

The same population was screened using single strand conformational polymorphism (SSCP) analysis to assay for the presence of SMN^T, following the protocol of Lefebvre et al. [1995]. This procedure was modified so that nonradioactive PCR products were separated on 12.5% acrylamide for 170 Vh at 10 mA using a PhastSystem™ (Pharmacia Biotech, Uppsala, Sweden), then visualized by silver staining (Fig. 1) [Aubry et al. 1995]. Banding patterns were compared with a subsample which had been resolved according to the procedure of Lefebvre et al. [1995] (radiolabelled products separated on mutation detection enhancement gel (MDE, J.T. Baker Inc., Phillipsburg, NJ) at 4°C for 18–24 h at 4 W) to verify consistency of deletion detection between methods.

One affected individual was assessed from each family, with or without parents (obligate carriers). These results were tabulated and combined with the frequencies of NAIP and SMN^T deletions that have been reported by others (Table I) [Lefebvre et al., 1995; Rodrigues et al., 1995; Cobben et al., 1995; Hahnen et al., 1995; Burlet et al., 1996; Wang et al., 1996].

Risk Calculations

To calculate a SMN/NAIP genotype-based risk for SMA, it is first necessary to establish a prior risk for the disorder. The prior risk of having SMA at birth or in early childhood, can be estimated from the frequency of SMA in that population. A fairly broad range of SMA birth incidence estimations have been postulated, from 1/6,720 in North Dakota [Burd et al., 1991] to 1/24, 100 in North-East England [Pearn, 1978]. A pooled frequency across seven different studies on populations from Europe and Canada probably provides the most reliable estimate: a birth incidence of 5/100,000 for type I SMA, and an early childhood prevalence of 4/100,000 for type II and III SMA [Emery et al., 1991]. The birth, or early childhood prevalence of all types of SMA would therefore be 9/100,000.

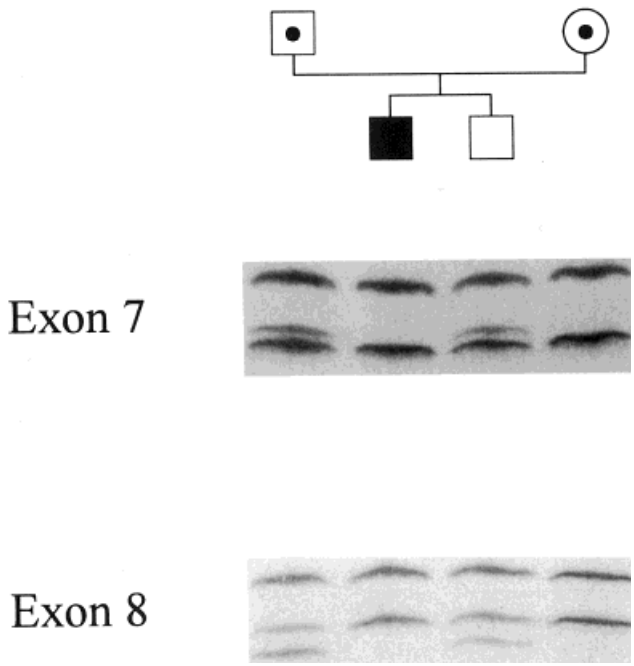

Fig. 1. Silver-stained acrylamide gels showing PCR products from SMN exons 7 and 8. The carrier father and unaffected son, both show the normal banding pattern. The carrier mother and affected son both show the affected banding pattern. The mother's pattern was consistent in 3 repetitions of sampling and analysis.

Calculation of the chromosomal frequencies of NAIP and SMN^T deletions is limited by the fact that these mutations can only be detected in the homozygous state (i.e., when both NAIP or SMN^T alleles have deletions). There are two reasons for this technical limitation: first, the test is PCR-based and therefore whether a positive result reflects one or more gene copies cannot be determined; and second, there are a variable number of copies of NAIP and SMN in each individual, some of which are functional and some of which are not. Consequently, heterozygous deletion carriers (one normal and one deleted functional allele) cannot be detected directly.

Assuming a random segregation of chromosomes, the frequency of deletions on known SMA chromosomes (ΔF) can be calculated from the number of SMA individuals with homozygous deletions (A), relative to the total number of SMA individuals tested (N).

TABLE I. Frequency of Homozygosity for NAIP Exon 5/Exon 5–6 Deletions (ΔNAIP), and for SMN^T Exon 7/Exon 7–8 Deletions (ΔSMN^T) in Individuals With SMA and Their Parents

	Type I	Type II/III	Parents	Source
	(Affected)	(Affected)	(Carrier)	
ΔNAIP	33/72	13/119	5/307	Hahnen et al. [1995]
	29/44	6/62	1/66	Burlet et al. [1996]
	26/50	15/94	4/225	Our findings
	88/166	34/275	10/598	Pooled data
ΔSMN^T	102/103[a]	124/126[a]		Lefebvre et al. [1995]
	50/51	87/89		Rodrigues et al. [1995]
	45/49	51/54		Cobben et al. [1995]
	69/72	103/119	1/348	Hahnen et al. [1995]
	42/44	60/62	0/66	Burlet et al [1996]
			2/214	Wang et al. [1996]
	44/50	79/94	1/225	Our findings
	352/369	504/544	4/853	Pooled data

[a] Assumed distribution of deletions given total of 226/229 for all SMA individuals.

$$\Delta F = \sqrt{\frac{A}{N}} \quad (1)$$

From this estimate, the conditional probability of SMA with and without deletions of SMN^T can be derived (see Appendix) and used for Bayesian analysis.

RESULTS

Data Compilation

ΔNAIP and ΔSMN^T genotype data from previous studies [Lefebvre et al., 1995; Rodrigues et al., 1995; Cobben et al., 1995; Hahnen et al., 1995; Burlet et al., 1996; Wang et al., 1996] were combined with our findings (Table I). We found that 52% of type I and 16% of type II and III SMA individuals did not contain NAIP exon 5. In addition, 4 of the 225 parents examined were homozygous for this NAIP deletion. In contrast, 88% of those with type I and 84% of those with type II and III SMA were deleted for exon 7 or exons 7 and 8 of the telomeric copy of SMN. Furthermore, 1 of the 225 obligate carrier parents tested was homozygous for the SMN^T exon 7 and 8 deletion, without the NAIP deletion (Fig. 1). This parent whose genotype was confirmed on retesting, was clinically unaffected, indicating the presence of two ΔSMN^T chromosomes not associated with the SMA phenotype.

Association of SMN^T and NAIP Deletions

All of the ΔNAIP SMA samples tested were also ΔSMN^T. There were, therefore, no affected ΔNAIP individuals that were not ΔSMN^T. The only samples that were ΔNAIP and not ΔSMN^T came from carrier parents. Since NAIP-deleted affected individuals were a subset of those that had the SMN^T deletion, ΔNAIP was not considered as an independent variable that modified the risk of SMA in ΔSMN^T-positive samples. Using pooled data (Table I), ΔNAIP occurred 4.29 times more frequently in type I cases than in type II and III cases. Homozygosity for NAIP and SMN^T deletions is, therefore, associated with an increased severity of SMA in an affected individual.

Bayesian Analysis

Assuming a birth and early childhood SMA prevalence of 9/100,000, it is possible to calculate prior risks for SMA in various subpopulations (Table IIA). The frequencies of SMN^T deletions in the pooled data were used to determine conditional probabilities for SMA. These calculations are outlined in the Appendix and summarized in Table IIB. By combining prior risk estimates with conditional probabilities, posterior probabilities for the presence of SMA with and without SMN^T deletions were determined (Table IIC). These results were applied to cases as outlined below.

Case Studies

A number of situations may be encountered in which SMN genotyping can be helpful in determining the risk of SMA. With regard to SMN and NAIP status, there are three possible outcomes for the patient: (1) no deletions, (2) SMN^T deletion alone, (3) NAIP and SMN^T deletions. We present several case studies (most of which have been encountered by our service laboratory) to illustrate the diagnostic application of these findings.

Case 1. Hypotonic infant with neither clear underlying SMA, nor previous family history of SMA. A common clinical problem is the birth of an infant with hypotonia [Dubowitz, 1980]. Among hypotonic newborn infants without underlying perinatal asphyxia, sepsis, or a metabolic disorder, spinal muscular atrophy is considered to be the most frequent cause (Dubowitz, personal communication). Without an exact figure available for SMA prior risk, a theoretical range of 50% to 99% can be assigned to these hypotonic infants (although clearly, a number closer to 50% than 99% would be expected), with carrier and noncarrier risks proportional to the general population risk (Table IIA). The ΔSMN^T genotype frequencies (Table IIB) can be combined with the prior risks to generate the a priori risks shown in Table IIC. If no deletions are found, there re-

TABLE II. Prior Risks, Conditional and Posterior Probabilities From Bayesian Estimates of the Likelihood That a Child Will Have Spinal Muscular Atrophy

A. Prior risk of SMA estimated in various populations

Contingencies for prior risk	Affected	Carrier	Normal
Hypotonic child/unknown parental status	0.500–0.990	0.0094–0.0002	0.4906–0.0098
Child of obligate carriers	0.25	0.50	0.25
Child of one obligate carrier, with confirmed SMA chromosome inheritance by linkage	0.0094	0.9906	
Child of one obligate carrier, without linkage data	0.0047	0.50	0.4953
Child in general population	9.00×10^{-5}	0.0188	0.981

B. Conditional probabilities for presence or absence of SMN^T exon 7.

Proportion of individuals with	Affected	Carrier	Normal
ΔSMN^T	0.9417	0.0047	2.34×10^{-5}
No ΔSMN^T	0.0583	0.9953	0.999977

C. Posterior probabilities of spinal muscular atrophy for individuals outlined in case studies, in presence or absence of the SMN^T deletions. Case 1 refers to a postnatal diagnosis, cases 2–4 refer to prenatal diagnoses.

Molecular test results	Case 1 Hypotonic infant (neuromuscular etiology)	Case 2 Offspring of obligate carrier parents	Case 3 Offspring of obligate carrier with partner of unknown status and: SMA chromosome by linkage	Case 3 Offspring of obligate carrier with partner of unknown status and: No linkage information	Case 4 Offspring of parents from general population
No ΔSMN^T	0.055–0.832	0.019	5.56×10^{-4}	2.76×10^{-4}	5.25×10^{-6}
ΔSMN^T	0.999	0.990	0.656[a]	0.653[a]	0.433[a]

[a] Risk of type I vs. type II/III SMA, is increased approximately 5-fold by coincident finding of ΔNAIP.

mains a theoretical range of 5% to 83% risk of SMA to the infant, making the test result of relatively little clinical utility in this situation. However, the deletion of SMN^T with or without NAIP deletions in a hypotonic infant makes it highly likely that the diagnosis is SMA (99.9%), whether a 50% or 99% prior risk is used. Since neonatal hypotonia is present, ΔSMN^T would be diagnostic for type I SMA and therefore, anticipated severity of the disease would not be modified by the additional finding of ΔNAIP.

Case 2. Prenatal testing in parents who have already had a child with SMA. The parents who are obligate carriers, with a prior risk of 25% for future pregnancies, opt for molecular testing on a subsequent pregnancy. This may occur with or without SMN/NAIP genotyping for the previous SMA child. If previous genotyping results are not available, using the prior risk for carrier parents from Table IIA, and SMN^T deletion data (Table IIB), Table IIC can be generated. Even without molecular results for the previous SMA-affected child, ΔSMN^T will confer a SMA risk of 99.0% to the patient. As in Case 1, ΔNAIP would not modify the risk conferred by ΔSMN^T, since SMA severity should be consistent with the previous affected child (for exceptions, see Discussion). The absence of a homozygous SMN^T deletion would reduce the risk of SMA to 1.9%. If previous genotyping results are available, the presence of the same result in the current pregnancy can be used for confirmation of SMA in the fetus [Rodrigues et al., 1995]. An exception to this generalization would be a pregnancy in which one of the parents is homozygous for the SMN^T deletion. In this instance, predictive testing based only on presence or absence of ΔSMN^T would be uninformative [Wang et al., 1996].

Case 3. Prenatal testing for a couple comprising a known carrier (either through a previous affected child, or through linkage analysis relative to an SMA sib) and an individual with unknown carrier status. In this situation, if molecular test results are not available for a previous affected child, the prior risk of SMA for the current pregnancy could be calculated as 0.25×0.0188 (population carrier frequency) $= 4.7 \times 10^{-3}$ (or about 0.5%; see Table IIA). This risk is influenced by the SMN genotype (Table IIB), to generate the probabilities of SMA depicted in Table IIC. The finding of a SMN^T deletion would result in a 65.3% risk of SMA. No deletion of SMN^T would result in a risk of .003%. If linkage data are available for the obligate carrier, and the SMA chromosome from that parent is known to be present in the fetus, then there are only two possible outcomes: the fetus will be a carrier or affected with SMA (Table IA). Bayesian analysis for these outcomes results in a slightly increased probability of SMA (Table IIC).

In this case, because one parent has an unknown SMA genotype, the presence of ΔNAIP with ΔSMN^T, in the current pregnancy, modifies the relative risk of the SMA phenotype. ΔNAIP is 4.29 times more common in type I than in type II/III SMA, and type I SMA is more common in the general population than type II/III SMA (5/100,000 vs. 4/1000,000). The chance of type I SMA is therefore increased by a factor of $4.29 \times 5/4 = 5.3$ relative to type II/III when ΔNAIP is detected with ΔSMN^T.

Case 4. Testing in the absence of a clinical diagnosis or family history of SMA. This may result from a referral without relevant information, or from prenatal testing in the absence of a family history. Although such situations are relatively uncommon, an approximation of the probability of SMA from deletion testing is possible. In these circumstances, the prior risk to the individual being tested would be the general population risk (Table IIA), and the conditional probabilities for presence or absence of ΔSMN^T would be the same as those used in the other calculations (Table IIB). The finding of no SMN^T deletion lowers the risk of SMA to the individual from 9/100,000 by a factor of approximately 17 to less than 1/190,000. The deletion of SMN^T exon 7 or 7 and 8 confers a 43% risk of SMA (see Table IIC). As in Case 3, the presence of ΔNAIP with ΔSMN^T increases the probability of type I vs. type II/III by a factor of approximately 5.

DISCUSSION

The data compiled by our group and others, when applied to individuals at risk for SMA, indicate that a molecular diagnosis of this disorder is feasible. A reasonable level of certainty (i.e., >95%) can be achieved in some populations. It is also possible in some cases to anticipate the relative severity of the SMA phenotype.

A positive test for SMN^T deletions in the offspring of obligate carrier parents or in hypotonic infants (with neuromuscular etiology) is associated with an extremely high probability of SMA (greater than 98%). In prenatal cases involving one confirmed carrier parent and a new partner, it has been theoretically possible to increase the risk of SMA to the fetus from 0.5% to approximately 65% in the presence of a SMN^T deletion, and to decrease the risk of SMA from 0.5% to 0.0005% in the absence of this deletion. From a clinical standpoint, the 43% risk of SMA to someone in the general population with a SMN^T deletion is somewhat academic, because this presumes a complete lack of phenotypic information, and would apply primarily to a situation where random screening of pregnancies was used.

Predictive testing for SMA is a useful application of molecular results, but in most situations there is also a desire to predict the severity of the disease (e.g., will this be a type I or a type III infant?). An individual who has SMA and is homozygous for the NAIP deletion is approximately five times more likely to have type I SMA than type II/III SMA. This finding implicates NAIP as disease-modifying, rather than a directly causative gene [Crawford and Pardo, 1996]. It should be noted that family history and clinical signs and symptoms must be considered in this interpretation. It has previously been observed that within a nuclear family, affected sibs of type II/III children usually have type II/III SMA. An exception to this pattern has been seen in affected males who can have a much more severe form of SMA than their female sibs [Dubowitz, 1964]. In all nuclear families that we analyzed with type II/III children homozygous for both NAIP and SMN^T deletions, all subsequent affected children were type II/III with homozygous NAIP and SMN^T deletions.

The range of phenotype associated with SMN^T deletions, implicates factors other than the presence of these mutations in the pathogenesis of SMA. Due to the highly repetitive nature of the DNA in the SMA region of chromosome 5(5q13), the number of copies of SMN and/or NAIP is variable, and an inverse correlation may exist between the number of copies of deleted genes and the severity of the SMA phenotype [Somerville et al., 1995]. One possible corollary of this model is that the size of the deletion is important, with a smaller deletion being present in milder forms of the disease [Wang et al., 1996]. This model is supported by the finding that type I SMA is more likely to be associated with NAIP and SMN deletions together, than is type II or III SMA. All parents homozygous for SMN^T deletions were in type II/III families, supporting the association between isolated SMN^T deletions and a milder or even subclinical phenotype. NAIP and SMN may actually form parts of a multimeric protein complex, or components of a functional pathway, so that deletions in both are phenotypically additive. It is also possible that neither gene has a direct involvement in SMA pathogenesis, but is in close proximity to the SMA gene, and deletions of SMN^T with or without NAIP are associated with deletions of the causative gene. While the precise molecular pathology underlying SMA remains uncertain, we conclude that our analysis helps to quantify the degree of SMA risk when deletions of SMN^T, with or without deletions of NAIP, are observed.

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REFERENCES

Aubry HL, MacKenzie AE, Surh LC (1995): Delineating the mutations in spinal muscular atrophy: Improved molecular detection and genotype-phenotype correlations. Am J Hum Genet 57(Suppl.): A234.

Burd L, Short SK, Martsolf JT, Nelson RA (1991): Prevalence of type I spinal muscular atrophy in north Dakota. Am J Med Genet 41:212–215.

Burlet P, Bürglen L, Clermont O, Lefebvre S, Viollet L, Munnich A, Melki J (1996): Large scale deletions of the 5q13 region are specific to Werdnig-Hoffmann disease. J Med Genet 33: 281–283.

Cobben JM, Van Der Steege G, Grootscholten P, De Visser M, Scheffer H, Buys CHCM (1995): Deletions of the survival motor neuron (SMN) gene in unaffected siblings of patients with spinal muscular atrophy (SMA). Am J Hum Genet 57:805–808.

Crawford TO, Pardo CA (1996): The neurobiology of childhood spinal muscular atrophy. Neurobiol Dis 3:97–110.

Dubowitz V (1964): Infantile muscular atrophy—a prospective study with particular reference to a slowly progressive variety. Brain 87:707–718.

Dubowitz V (1980): "The Floppy Infant." (2nd ed.) Lavenham, Suffolk, UK: The Lavenham Press Ltd.

Emery AEH (1991): Population frequencies of inherited neuromuscular diseases—a world survey. Neuromusc Dis 1:19–29.

Hahnen E, Forkert R, Marke C, Schöneborn Sr, Schönling J, Zerres K, Wirth B (1995): Molecular analysis of candidate genes on chromosome 5q13 in autosomal recessive spinal muscular atrophy: Evidence of homozygous deletions of the SMN gene in unaffected individuals. Hum Mol Genet 4:1927–1933.

Lefebvre S, Bürglen L, Reboullet S, Clermont O, Burlet P, Viollet L, Benichou B, Cruard C, Millasseau P, Zeviani M, Le Paslier D, Weissenbach J, Munnich A, Melki J (1995): Identification and characterization of a spinal muscular atrophy-determining gene. Cell 80:155–165.

Liston P, Roy N, Tamai K, Lefebvre C, Baird S, Horvat GC-, Farahani R, McLean M, Ikeda J-E, Mackenzie A, Korneluk RG (1996): Suppression of apoptosis in mamalian cells by NAIP and a related family of IAP genes. 379:349–353.

Nakahori Y, Hamano K, Iwaya M, Nakagome Y (1991): Sex identification by polymerase chain reaction using X-Y homologous primers. Am J Med Genet 39:472–473.

Pearn J (1978): Incidence, prevalence, and gene frequency studies of chronic childhood spinal muscular atrophy. J Med Genet 15:409–413.

Rodrigues NR, Owen N, Talbot K, Ignatius J, Dubowitz V, Davies KE (1995): Deletions in the survival motor neuron gene on 5q13 in autosomal recessive spinal muscular atrophy. Hum Mol Genet 4:631–634.

Roy N, Mahadevan MS, McLean M, Shutler G, Yaraghi Z, Farahani R, Baird S, Johnston AB-, Lefebvre C, Kang X, Salih M, Aubry H, Tamai K, Guan X, Ioannou P, Crawford TO, de Jong PJ, Surh L, Ikeda J-E, Korneluk RG, MacKenzie A (1995): The gene for neuronal apoptosis inhibitory protein is partially deleted in individuals with spinal muscular atrophy. Cell 80:167–178.

Schöneborn SR, Forkert R, Hahnen E, Wirth B, Zerres K (1996): Clinical spectrum and diagnostic criteria of infantile spinal muscular atrophy: Further delineation on the basis of SMN deletion findings. Neuropediatrics 27:1–8.

Somerville MJ, Mahadevan MS, Korneluk RG, MacKenzie AE, Surh LC (1995): Dosage of candidate genes and severity of spinal muscular atrophy. Am J Hum Genet 57(Suppl.): A251.

Wang CH, Xu J, Carter TA, Ross BM, Dominski MK, Bellcross CA, Penchaszadeh GK, Munsat TL, Gilliam TC (1996): Characterization of survival motor neuron (SMN^T) gene deletions in assymptomatic carriers of spinal muscular atrophy. Hum Mol Genet 5:359–365.

Zerres K, Schöneborn SR (1995): Natural history in proximal spinal muscular atrophy. Arch Neurol 52:518–523.

APPENDIX
Calculation of Conditional Probabilities

The weighted average frequency of candidate gene deletions on type I, II, and III SMA chromosomes (ΔF_s) is a function of the prevalence of type I (p_1) and type II/III (p_{23}) SMA relative to those homozygous deleted type I (A_1) and type II/III (A_{23}) SMA individuals out of the total number of type I (N_1) and type II/III (N_{23}) SMA individuals tested.

$$\Delta F_s = \left(\frac{p_1}{p_1 + p_{23}} \cdot \sqrt{\frac{A_1}{N_1}}\right) + \left(\frac{p_{23}}{p_1 + p_{23}} \cdot \sqrt{\frac{A_{23}}{N_{23}}}\right) \quad (2)$$

The determination of a deletion frequency for non-SMA chromosomes from the pooled analysis of obligate SMA heterozygote parents is somewhat more complicated. Given that our assay detected only deletion homozygotes (and not heterozygotes), it is evident that in the parents, we are assaying the status of the non-SMA chromosome only in those individuals who also carry a deleted SMA chromosome. Therefore, the frequency of chromosomes that are apparently non-SMA (normal) but deleted for candidate genes (ΔF_n) is calculated from the proportion of those carrier parents with homozygous deletions (A_p) out of the total number of parents tested (N_p) relative to the average frequency of candidate gene deletions on type I, II, and III SMA chromosomes (ΔF_s)

$$\Delta F_s \cdot \Delta F_n = \frac{A_p}{N_p} \qquad \therefore \Delta F_n = \frac{A_p}{N_p \cdot \Delta F_s} \quad (3)$$

Nondeleted SMA (F_s) and non-SMA (F_n) chromosomes comprise the remainder of those observed. Therefore, $F_s = 1 - \Delta F_s$ and $F_n = 1 - \Delta F_n$. Conditional probabilities for presence or absence of deletions in SMA-affected, carrier, and normal individuals can be calculated from the ΔF_s, F_s, ΔF_n, and F_n values.

For individuals with SMA, the probability of finding a homozygous ΔSMN^T (ΔS_a) is; $\Delta S_a = (\Delta F_s)^2$, and the probability of finding no homozygous deletion (S_a) is;

$$S_a = (F_s)^2 + 2(\Delta F_s \cdot F_s) = 1 - \Delta S_a$$

For SMA carriers, the probability of finding a homozygous ΔSMN^T (ΔS_c) is; $\Delta S_c = (\Delta F_n \cdot \Delta F_s)$ and the probability of finding no homozygous deletion (S_c) is;

$$S_c = (\Delta F_n \cdot F_s) + (F_n \cdot F_s) + (F_n \cdot \Delta F_s) = 1 - \Delta S_c$$

For homozygous unaffected individuals, the probability of finding a homozygous ΔSMN^T (ΔS_n) is; $\Delta S_n = (\Delta F_n)^2$ and the probability of finding no homozygous deletion (S_n) is;

$$S_n = (F_n)^2 + 2(\Delta F_n \cdot F_n) = 1 - \Delta S_n$$

With the estimation of prior risk for SMA, as well as ΔS_a, S_a, ΔS_c, S_c, ΔS_n, and S_n values for SMN, Bayesian analyses can be used to obtain probabilities for SMA in the presence or absence of these deletions.

Application of Equations to Compiled Data

Using pooled data (Table I) the ΔSMN^T frequency of SMA chromosomes is:

$$\Delta F_s = \left(\frac{5/100,000}{9/100,000} \cdot \sqrt{\frac{352}{369}}\right) + \left(\frac{4/100,000}{9/100,000} \cdot \sqrt{\frac{504}{544}}\right) = \mathbf{0.9704}$$

using this value, $\Delta F_n = \dfrac{4}{(853 \cdot 0.9704)} =$ **0.004832.**

Therefore, $F_s = 1 - 0.9704 =$ **0.0296**, and similarly, $F_n = 1 - 0.004832 =$ **0.995168**.

The following SMN conditional probabilities

$\Delta S_a = (0.9704)^2 =$ **0.9417**
$S_a = 1 - \Delta S_a =$ **0.0583**

$\Delta S_c = (0.004832 \cdot 0.9704) =$ **0.0047**
$S_c = 1 - \Delta S_c =$ **0.9953**

$\Delta S_n = (0.004832)^2 =$ **2.34×10^{-5}**
$S_n = 1 - \Delta S_n =$ **0.99997**

were therefore employed in Bayesian analyses (Table IIB).